Macro Paper Discussion: Nov 15, 2013 “Structural and Energetic Mechanisms of Cooperative Autoinhibition and Activation of Vav1” Yu et al, Cell 2010

Background: Vav1 is a GEF for Rho family GTPases. Its core DH domain binds the GDP form of GTPase to catalyze GDP exchange for GTP. This interaction is blocked because the protein interaction surface of the DH domain that binds GTPase is occluded by an autoinhibitory helix. Phosphorylation of this helix results in a disorder-to-order transition, effectively melting the helix and revealing the GTPase binding surface on the DH domain. An apparent paradox in the NMR structure we discussed last Friday is that the key phosphorylation site (Y174) is buried in the autoinhibited structure, raising the question of how kinases gain accesss to the site. This week we discussed a paper by Li et al (NSMB, 2008) showing that the autoinhibited structure is energetically stable ground state that can fluctuate into an excited state that allows access for the kinase. A series of mutants were made that destabilize this ground state, increasing the population of open form. The catalytic efficiency of phosphorylation correlated with population of open form. This analysis revealed the population of open state for wild-type is just under 10% and that autoinhibition suppressed GEF activity only 10-fold.

The current paper addresses how additional domains of Vav1 can work together to stabilize the closed form contributing another 10-fold to repression of activity. The construct used for crystallization, NMR and biochemistry has 5 domains, in order from N-to-C-term: the calponin homology domain (CH), the Acidic domain (Ac) the catalytic DH domain, the PH domain and a Zinc-finger (ZF). Importantly, this 5 domain protein is globular and appears well folded by crystallography-the DH domain maintains interactions with the autoinhibitory helix of the Ac domain but additional interactions are revealed in the structure that correlate with an addditinal 10-fold repression of Vav1’s GEF activity. This is important biologically because deletion of other domains (such as the calponin homology domain ) increase the transforming activity of Vav1. A major questions is: how do multi-domain proteins fold in order to achieve robust autoinhbition but at the same time enable rapid activation by post-translational modifications.

Questions:

Figure 1
1) How many tyrosine phosphorylation sites are in Vav1?
2) What is the evidence the CH domain and other the tyrosine in the Ac domain are important?

Figure 2:
3) How much is Vav1 inhibited by additional modulatory domains (those outside the Ac-DH construct used for NMR)?
4) How is this inhibition measured?
5) Is there a correlation between enhanced activity of Vav1 and binding to the GTPase Rac (Figure 2B and C)? Why?
Figure 3
5) Based on the structure, GEF activity and NMR data in the figure, how do you think the modulatory domains further repress activity? What is the significance of the three-way domain interfaces depicted in Figure 3?

Figure 4
4) What is the evidence for interdomain cooperativity? How are NMR and activity assays used to support this point?

Figure 5 Why does adding the CH domain in “trans” inhibit GEF activity, but only if the AC domain is intact and fused to DH?

Figure 6 What observations suggest the N-terminal phosphosites prime phosphorylation of the Ac-inhibitory helix harboring Y174?

How general are these phenomenon in signaling? A comparison of regulatory transitions in other signaling molecules and small molecular inhibitors that bind and stabilize specific substates could be interesting. Eg-kinases and type-II inhibitors such as imatinib (Gleevec).